

Attorney Docket No.: **DEX-0267**
Inventors: **Salceda et al.**
Serial No.: **10/001,843**
Filing Date: **November 20, 2001**
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Amendments to the specification:

Please insert the following paragraphs at page 118, line 40:

Suppression subtractive hybridization (Clontech PCR-SELECT)

Clontech PCR-SELECT is a PCR based subtractive hybridization method designed to selectively enrich for cDNAs corresponding to mRNAs differentially expressed between two mRNA populations (Diatchenko *et al*, *Proc. Natl. Acad. Sci. USA*, Vol. 93, pp. 6025-6030, 1996).

Clontech PCR-SELECT is a method for enrichment of differentially expressed mRNAs based on a selective amplification. cDNA is prepared from the two mRNA populations which are to be compared (Tester: cDNA population in which the differentially expressed messages are sought and Driver: cDNA population in which the differentially expressed transcripts are absent or low). The tester sample is separated in two parts and different PCR adapters are ligated to the 5' ends. Each tester is separately annealed to excess driver (first annealing) and then pooled and again annealed (second annealing) to excess driver. During the first annealing, sequences common to both populations anneal. Additionally the concentration of high and low abundance messages are normalized since annealing is faster for abundant molecules due to the second order kinetics of hybridization. During the second annealing cDNAs unique or overabundant to the tester can anneal together. Such molecules have different adapters at their ends. The addition of additional driver during the second annealing enhances the enrichment of the desired differentially expressed sequences. During subsequent PCR, molecules that have different adapters at each end amplify exponentially. Molecules which have identical

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adapters, or adapters at only one end, or no adapters (driver sequences) either do not amplify or undergo linear amplification. The end result is enrichment for cDNAs corresponding to differentially expressed messages (unique to the tester or upregulated in the tester). This technique was used to identify transcripts unique to breast tissues or messages overexpressed in breast cancer tissues.

Pairs of matched samples were isolated from the same patient, a cancer sample, and the "normal" adjacent tissue from the same tissue type. The mRNA from the cancer tissue was used as the "tester", and the non-cancer mRNA as a "driver". The non-cancer "driver" is from the same individual and tissue as the cancer sample (Matched). Alternatively, the "driver" can be from a different individual but the same tissue as the tumor sample (unmatched). In some cases "driver" mixtures of mRNAs derived from non-cancer tissues types different from the cancer tissue type are used. The last approach allows the identification of transcripts whose expression is specific or upregulated in the cancer tissue type analyzed. Such transcripts may or may not be cancer specific in their expression.

Several subtracted libraries were generated for breast tissue. The product of the subtraction experiments was used to generate cDNA libraries. These cDNA libraries contain Expressed Sequence Tags (ESTs) from genes that are breast specific, or upregulated in breast tissue. Randomized clones picked from each cDNA PCR Select library were sequenced and the genes identified by a systematic analysis of the sequence data against the LIFESEQ Gold database available from Incyte Pharmaceuticals, Palo Alto. The sequences with no significant homology to any DNA sequence present in the

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database are considered novel sequences. These breast cancer specific markers are DEX0120_1 through DEX0120_99 and correspond to markers of the instant sequence listing. In particular, SEQ ID NO:19 and 20 correspond to DEX0120_16 (SEQ ID NO:16).